# MODIFICATION BY TRICYCLIC ANTIDEPRESSANTS OF CORTICAL EEG CHANGES INDUCED BY CLONIDINE IN CONSCIOUS RATS

MILIND P. PARALE, USHA NAYAR* AND S. K. KULKARNI**

*Pharmacology Division,*
*Department of Phamaceuical Scienes,*
*Punjab University, Chandigarh - 160 014*
and
**Department of Physiology,*
*All India Institute of Medical Sciences,*
*Ansari Nagar, New Delhi - 100 029*

( Received on September 7, 1985 )

**Summary** : The effects of various tricyclic antidepressants on clonidine-induced electroencephalographic changes were investigated in rats. The EEG pattern of conscious rats was recorded by means of bipolar electrodes, implanted chronically. Clonidine (50, 150 and 300 $\mu g/kg$) not only synchronizcd cortical EEG pattern but also evoked signs of behavioural depression within 15 min of its administration. Pretreatment with imipramine, desipramine, trimipramine, amitriptyline, nortriptyline and doxepin reduced clonidine-induced EEG synchrony without showing any effects per se. Acute treatment with tricyclic antidepressants failed to modify but, chronic treatment abolished the clonidine - induced behavioural depressive signs. Chronic administration of tricyclic antidepressants (10 $mg/kg/day$) evoked more pronounced antagonism of the EEG effects of clonidine. Yohimbine (200 $\mu g/kg$) pretreatment inhibited both, clonidine-induced EEG synchrony and behavioural effects. Guanfacine as well as B-HT 920. elicited clonidine - like effects on cortical EEG pattern and behaviour. The present data suggests that antagonism of clonidine-induced EEG synchronization in conscious animals could serve as a useful test for screening of antidepressant drugs.

**Key words** : clonidine B-HT 920 guanfacine
tricyclic antidepressants cortical EEG

## INTRODUCTION

Clonidine, an alpha$_2$ adrenoceptor agonist, produces numerous symptoms of behavioural depression in laboratory animals. It induces sedation (2), antinociception

**Corresponding Author

(12) and hypothermia (9); alleviates alcohol abstinence signs (13) and suppresses exploratory (8), avoidance (15) and operant behaviour (4) in rats, mice and the Mongolian gerbil. Furthermore, Florio *et al.* (6) have demonstrated that the behavioural depression induced by clonidine in rats is accompanied by EEG synchronization. Recently antidepressant drugs particularly when given chronically have been reported to attenuate various effects of clonidine viz. sedation (7), hypothermia (19), reduction of noradrenaline turnover (3) and behavioural depression (8). In the present study we have investigated the acute and chronic effects of tricyclic antidepressants on the cortical EEG changes induced by clonidine. Further, we have investigated whether the more specific $\alpha_2$-adrenoceptor agonists such as B-HT 920 (11) and guanfacine (1) also evoke clonidine-like EEG synchronization or not.

## MATERIAL AND METHODS

Male Wistar rats weighing 200-250 g were employed for the study. They were supplied with food and water *ad lib.* Under pentobarbitone (40 *mg/kg*, ip) anaesthesia, two screws with connecting leads were fixed on both frontal regions (2 mm anterior to the coronal suture and 4 *mm* lateral to the mid-sagittal suture) of the rat with dental cement taking necessary aseptic precautions. The animals were housed individually in separate cages for 5-7 days to provide for recovery from surgical trauma. Each animal was acclimatized to the recording room for 24 h preceding an experiment and electroencephalographic recordings were done during the same time of the day (10 a.m.-2 p.m.). The bipolar electrodes from the rat were connected by means of a cable to a Grass Model 7D polygraph (Grass Instruments Co., Quincy, Mass, U.S.A.); permitting free movement of the rat within the home cage. The direct and integrated EEG signals were recorded on a chart paper (paper speed, 5 mm/sec) with the help of a pen oscillograph. A timer providing pips of 1 sec, 5 sec and 1 min was also used. The changes in the EEG pattern were recorded continuously in control as well as test groups. Each group consisted of a minimum of 5 animals. The drugs were either dissolved in normal saline or, if insoluble, dispersed in a suspension of carboxymethylacellulose (0.2% w/v). They were injected ip in a constant volume of 1 *ml*/100 *g*. The dose selection of various drugs was based on our earlier studies (12, 13). The doses correspond to pure bases of $\alpha$2-agonists and the salts of various other drugs. Yohimbine was administered 15 min and various tricyclics, 30 min before clonidine administration. Chronic schedule of tricyclics consisted of a daily injection of 10 *mg/kg/day* for eight successive days. Clonidine was administered 30 min after the last injection of the tricyclic antidepressant on the 8th day.

*Drugs:* The drugs used in the present study were obtained from following drug houses. Clonidine HCl (SG Pharmaceuticals, Baroda), guanfacine HCl (Sandoz Ltd., Basle, Switzerland), B-HT 920 HCl (Boehringer Ingelheim, W. Germany), yohimbine HCl

(E. Merck), imipramine HCl (SG Pharmaceuticals, Baroda), desipramine HCl (Ciba-Geigy), trimipramine HCl (May and Baker), amitriptyline HCl (MSD), nortriptyline HCl (Carter-Wallace, Panjim) and doxepin HCl (Torrent Lab., Ahmedabad). Doses refer to the salts.

*Statistics :* Statistical analysis was done by employing Mann-Whitney U (two-tailed) -test.

## RESULTS

Naive rats showed a desynchronized EEG pattern (Fig. 1). Clonidine (50 $\mu g/kg$. ip) synchronized this EEG pattern within 15 min of its administration and the effect lasted for

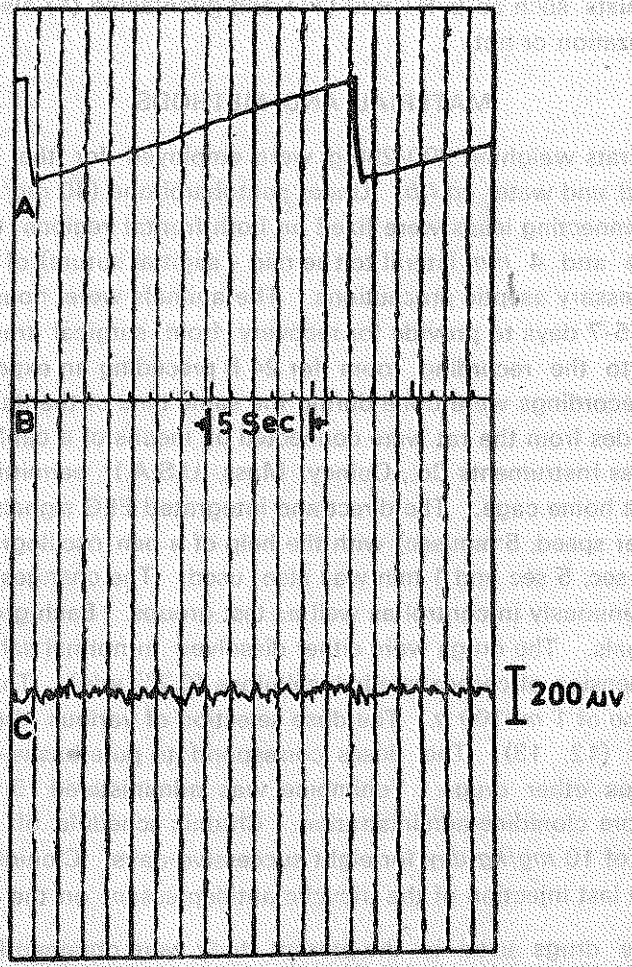


Fig. 1 : Desynchronized EEG pattern observed in control rats. (A : Integrated EEG, B ; Time calibration, C ; Original EEG).

about 75 min, whereas, normal saline did not have any influence on cortical EEG. Higher doses of clonidine (150, 300 *μg/kg*) evoked a dose dependent increase in EEG synchrony as manifested by increase in voltage output (Fig. 2) and the number of integrator resets occuring per unit time (Table I). In addition to its EEG effect, clonidine (150, 300 *μg/kg*) also produced apparent behavioural changes marked by drowsiness and signs of depression. The animals remained calm and passive in a crouched posture, confining to a corner of the cage after clonidine treatment. Various tricyclic antidepressants, such as imipramine, desipramine, trimipramine, amitriptyline, nortriptyline and doxepin did not have any effect *per se* on cortical EEG. However, clonidine-induced EEG synchrony was reduced after pretreatment with these tricyclics (Fig. 3), though, behavioural effects of clonidine remained unchanged. Chronic administration (10 *mg/kg/day*)

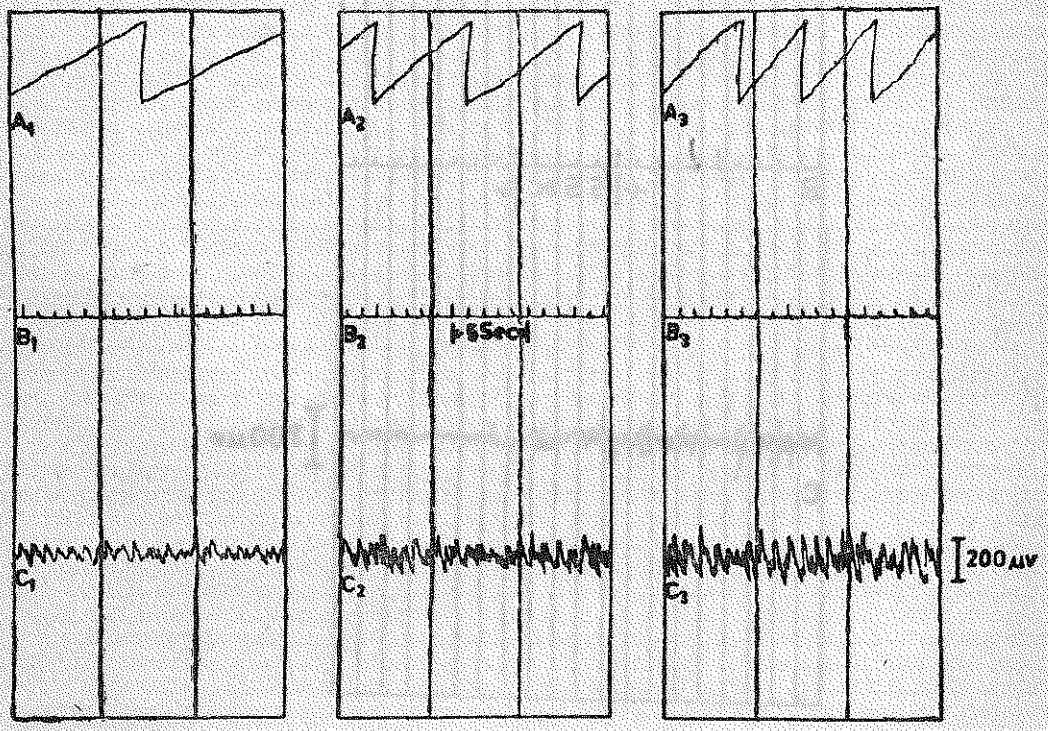


Fig. 2 : Effects of various doses of clonidine on the EEG pattern of the rat. (A : Integrated EEG, B : Time calibration, C : Orjginal EEG, 1, 2 and 3 represent the effects of 50, 150 and 300 *μg/kg* of clonidine, respectively).

of tricyclic antidepressants completely antagonized the EEG effects of clonidine (Table I) and the animals showed normal desynchronized EEG pattern when challenged with clonidine (Fig. 4). The signs of behavioural depression were abolished after chronic treatment with tricyclic antidepressants. Yohimbine (200 $\mu g/kg$) pretreatment inhibited

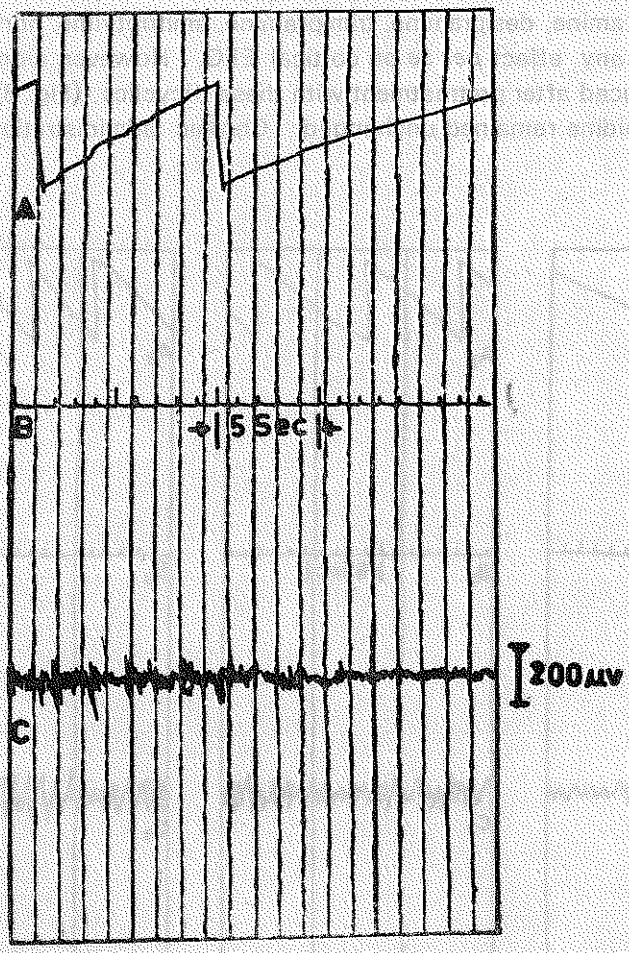


Fig. 3 : Effect of imipramine (10 *mg/kg*, ip) pretreatment on clonidine-induced EEG synchrony (A : Integrated EEG, B : Time calibration, C : Original EEG).

both, clonidine-induced EEG synchrony and behavioural effects. Guanfacine and B-HT 920, the other two alpha$_2$ adrenoceptor agonists elicited clonidine - like effects on behaviour and cortical EEG pattern (Table I).

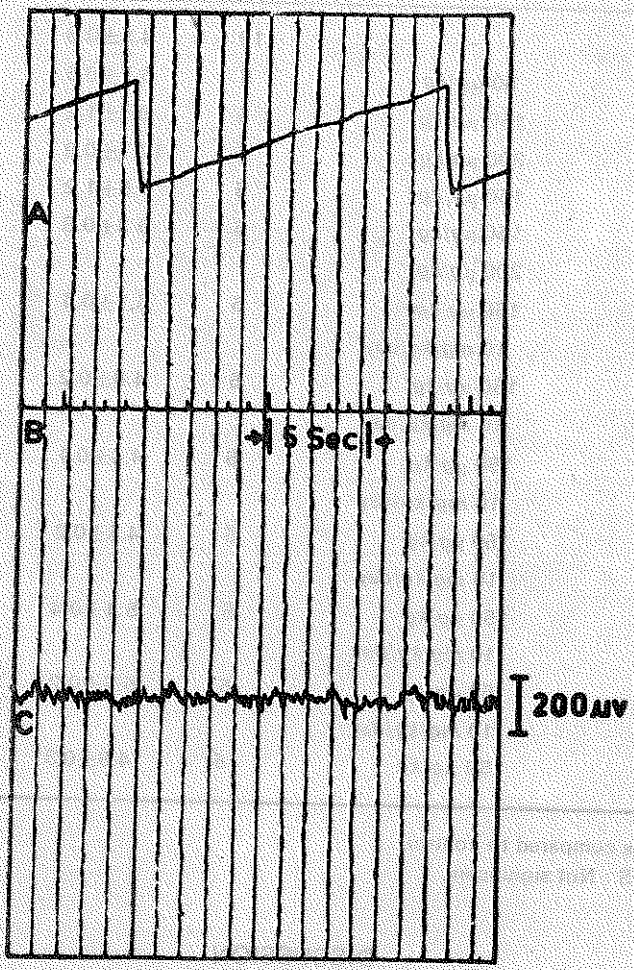


Fig. 4 : Modification by imipramine, administered chronically for 8 days (10 *mg/kg/day*) of clonidine-induced EEG changes. (A : Integrated EEG, B : Time calibration, C : Original EEG).

TABLE I : Effect of 8 days chronic treatment with various tricyclic antidepressants on the occurence of EEG integrator resets (an index of voltage output) enhanced by clonidine treatment.

| *Treatment* | *Dose, ip* | *n* | *Integrator resets per min. (Mean±SE)* | *P** |
|---|---|---|---|---|
| Control | — | 10 | 4.9±0.3 | — |
| Clonidine | 50 *μg/kg* | 5 | 6.7±0.5 | <0.01 |
| | 150 *μg/kg* | 5 | 9.7±1.2 | <0.002 |
| | 300 *μg/kg* | 5 | 12.7±0.9 | <0.002 |
| Guanfacine | 150 *μg/kg* | 5 | 9.5±1.2 | <0.002 |
| B-HT 920 | 150 *μg/kg* | 5 | 9.3±0.8 | <0.002 |
| Yohimbine | 200 *μg/kg* | | | |
| + clonidine | 150 *μg/kg* | 5 | 5.1±0.4 | NS |
| Imipramine | 10 *mg/kg/day* | | | |
| + clonidine | 150 *μg/kg* | 5 | 4.8±0.2 | NS |
| Desipramine | 10 *mg/kg/day* | | | |
| + clonidine | 150 *μg/kg* | 5 | 4.4±0.1 | NS |
| Trimipramine | 10 *mg/kg/day* | | | |
| + clonidine | 150 *μg/kg* | 5 | 4.5±0.2 | NS |
| Amitriptyline | 10 *mg/kg/day* | | | |
| + clonidine | 150 *μg/kg* | 5 | 5.1±0.4 | NS |
| Nortriptyline | 10 *mg/kg/day* | | | |
| + clonidine | 150 *μg/kg* | 5 | 5.3±0.2 | NS |
| Doxepin | 10 *mg/kg/day* | | | |
| + clonidine | 150 *μg/kg* | 5 | 4.8±0.3 | NS |

*As compared to control;
NS : Not significant.

## DISCUSSION

Recently alpha adrenoceptors have been classified into alpha$_1$ and alpha$_2$ subtypes (17, 20). The receptors more sensitive to phenylephrine than clonidine and more susceptible to blockade by prazosin than yohimbine have been designated as alpha$_1$ and those at which the effectiveness of these drugs is in reverse order have been designated

as alpha$_2$ adrenoceptors. Presynaptic alpha$_2$ adrenoceptors have been found to play an important role in the regulation of neurotransmitter release from noradrenergic nerve terminals (10). In the present study, clonidine, at micromolar doses, synchronized cortical EEG pattern of rats in a dose dependent manner. This effect of clonidine, was antagonized by yohimbine pretreatment thereby suggesting that it is an alpha$_2$ adrenoceptor mediated effect. This finding is in line with that of Florio *et al.* (6). Furthermore, our observation that chronic treatment with imipramine antagonized the EEG synchronizing effect of clonidine is consistent with the studies of Passarelli and Scotti de Carolis (14).

Clonidine evoked signs of behavioural depression in rats in the present study. This finding confirms the work of other investigators, who have shown that clonidine induces behavioural depression uniformly in rats mice and the Mongolian gerbil (2, 4, 7, 8, 12, 18). Recent studies have shown that chronic administration of antidepressants produce compensatory changes in noradrenergic neurotransmission (5, 8, 16). Spyraki and Fibiger (16) have reported that 15-day but not 2-day pretreatment with desipramine significantly reduced the effect of clonidine on exploratory behaviour. Whereas, Kostowski and Malatynska (8) reported that a 5-day treatment with antidepressants was sufficient to antagonize the depressant effect of clonidine on locomotor and exploratory activity. Our results indicate that chronic treatment of 8 days with tricyclic antidepressants was essential to produce compensatory changes in noradrenergic neurotransmission counteracting the clonidine effect.

Acute as well as chronic treatment with tricyclic anti-depressants inhibited clonidine-induced EEG synchrony This inhibitory effect of tricyclics may be a consequence of enhanced noradrenergic activity resulting from either inhibition of norepinephrine uptake and/or anticlonidine effect via interaction with presynaptic alpha$_2$ adrenoceptors. This speculation is in parallel with the recent studies of Finberg and Tal (5) ; showing down-regulation of presynaptic $\alpha_2$-adrenoceptors by chronic treatment with imipramine - like drugs.

In conclusion, we suggest that the antagonism of clonidine-induced EEG synchronization in conscious animals could serve as a valuable test for screening of agents with antidepressant potential.

## ACKNOWLEDGEMENTS

The skilful assistance of Dr. Pradip Mutalik in EEG recordings is gratefully acknowledged. This study was supported by University Grants Commission, New Delhi. Milind Parale is a UGC Fellow.

## REFERENCES

1. Dauses, J.P., A. Cardot, and P. Meyer. Specific binding of 3H-quanfacine to $\alpha_2$-adrenoceptors in rat brain : Comparison with clonidine. *J. Pharmacol., Paris*, **14** : 35-46, 1983.

2. Drew, G.M., A.J. Gower and A.S. Marriott. $\alpha_2$-adrenoceptors mediate clonidine - induced sedation in the rat. *Br. J. Pharmacol.*, **67** : 133-141, 1979.

3. Dubocovich, M.L., S.Z. Langer and F. Pelayo. Cocaine and desipramine antagonize the clonidine-induced inhibition of [3H]-noradrenaline release from the rat cerebral cortex. *Br. J. Pharmacol.*, **67** : 417-418, 1979.

4. Dwoskin, L.P. and S.B. Sparber. Comparison of yohimbine, mianserin, chlorpromazine and prazosin as antagonists of the suppressant effect of clonidine on operant behavior. *J. Pharmacol. Exp. Ther.*, **226** : 57-64, 1983.

5. Finberg, J.P.M. and A. Tal. Reduced peripheral presynaptic adrenoceptor sensitivity following chronic antidepressant treatment in rats. *Br. J. Pharmacol.*, **84** : 609-617, 1985.

6. Florio, V., L. Biauchi and V.G. Longo. A study of the central effects of sympathomimetic drugs : EEG and behavioural investigations on clonidine and naphazoline. *Neuropharmacology*, **14** : 707-714, 1975.

7. Gower, A.J. and A.S. Marriott. The inhibition of clonidine-induced sedation in the mouse by antidepressant drugs. *Br. J. Pharmacol.*, **69** : 287-288, 1980.

8. Kostowski, W. and E. Malatynska. Antagonism of behavioural depression produced by clonidine in the Mongolian gerbil : a potential screening test for antidepressant drugs. *Psychopharmacology*, **79** : 203-208, 1983.

9. Kulkarni, S.K. Apomorphine hypothermia : Interaction with serotonergic agents. *Pol. J. Pharmacol. Pharm.*, **32** : 15-20, 1980.

10. Langer, S.Z. Presynaptic regulation of catecholamine release. *Biochem. Pharmacol.*, **23** : 1793-1800, 1974.

11. Mottram, D.R. Pre-junctional $\alpha_2$-adrenoceptor activity of B-HT 920. *J. Pharm. Pharmacol.*, **35** : 652-655, 1983.

12. Parale, M.P. and S.K. Kulkarni. Apparent $pA_2$ estimates for antinociceptive receptor. *Arch. Int. Pharmacodyn.*, **275** : 59-64, 1985.

13. Parale, M.P. and S.K. Kulkarni. Studies with alpha$_2$ adrenoceptor agonists and alcohol abstinence syndrome in rats. *Psychopharmacology*, In Press, 1985.

14. Passarelli, F. and A. Scotti de Carolis. Effects of chronic treatment with imipramine, trazodone and electroshock on the behavioural and electroencephalographic modifications induced by clonidine in the rat. *Neuropharmacology* **22** : 785-789, 1983.

15. Robson, R.D., M.J. Antonaccio, J.K. Saelens and J. Liebman. Antagonism by mianserin and classical $\alpha$-adrenoceptor blocking drugs of some cardiovascular and behavioural effects of clonidine. *European J. Pharmacol.*, **47** : 431-442, 1978.

16. Spyraki, C. and H.C. Fibiger. Functional evidence for subsensitivity of noradrenergic $\alpha_2$-receptors after chronic desipramine treatment. *Life Sci.*, **27** : 1863-1867, 1980.

17. Starke, K. Alpha adrenoceptor subclassification. *Rev. Physiol. Biochem. Pharmacol.*, **88** : 199-236, 1981.

18. VanDerLaan, J.W., W. Van Veenendaal, P. Voorthuis, G. Weick and F.C. Hillen. The effects of centrally acting adrenergic agonists on temperature and on explorative and motor behaviour. Relation with effects on quassi-morphine withdrawal behaviour. *European J. Pharmacol.*, **107** : 367-373, 1985.

19. Voigtlander, P.F. von, H.J. Triezenberg and E.G. Losey. Interactions between clonidine and antidepressant drugs : A method for identifying antidepressant-like agents. *Neuropharmacology*, **17** : 375-381, 1978.

20. Wikberg, J. Differentiation between pre- and post-junctional alpha-receptors in quinea pig ileum and rabbit aorta. *Acta physiol. Scand.*, **103** : 225-239, 1978.